

In re Application of:

Prussak et al.

Application No.: 10/006,305

Filed: December 6, 2001

Page 2

PATENT

Attorney Docket No.: ST-UCSD3140

**In the Claims:**

Please amend claims 2 and 8 and add new claims 76-79 as provided below in the Listing of Claims.

The following Listing of Claims supersedes all prior listings of claims submitted in this application.

**Listing of Claims:**

1. (Cancelled)

2. (Currently amended) A nucleic acid molecule encoding a chimeric TNF $\alpha$   $\alpha$  ligand polypeptide having a Domain III and a Domain IV, wherein:

(a) the Domain III comprises a CD154 fragment lacking a metalloproteinase cleavage site present in wild-type CD154; and,

(b) the Domain IV comprises a TNF $\alpha$  fragment that binds to a TNF receptor;

wherein the encoded chimeric polypeptide is more resistant to cell membrane cleavage into soluble TNF $\alpha$  than are native TNF $\alpha$  and TNF $\alpha$  lacking a mmp cleavage site between Val77 and Pro88 of native TNF $\alpha$ .

~~, comprising a first polynucleotide encoding a Domain III fragment of CD154 lacking a metalloproteinase cleavage site present in Domain III of the wild type CD154 molecule, and a second polynucleotide encoding a Domain IV fragment of TNF $\alpha$  protein that binds to a TNF $\alpha$  receptor.~~

3. (Previously Presented) The nucleic acid molecule of claim 2 further comprising a third polynucleotide that encodes Domain II fragment of CD154.

4. (Previously Presented) The nucleic acid molecule of claims 2 or 3, further comprising a fourth polynucleotide that encodes a Domain I fragment of CD154.

5-7. (Cancelled)

8. (Currently amended) The nucleic acid molecule of claim 2, wherein the second polynucleotide encodes a Domain IV fragment of native TNF $\alpha$  that lacks a cleavage site of TNF $\alpha$  a protein.

9-10. (Cancelled)

11. (Previously Presented) The nucleic acid molecule of claim 2 further comprising a linker domain encoding a peptide of at least one amino acid that links the first polynucleotide to the second polynucleotide.

12. (Previously Presented) The nucleic acid molecule of claim 2, comprising a nucleotide sequence consisting of SEQ.ID. NO. 1.

13. (Cancelled)

14. (Withdrawn) A chimeric TNF $\alpha$ , comprising a Domain III fragment of a tumor necrosis factor ligand other than TNF $\alpha$  lacking a matrix metalloproteinase cleavage site and a Domain IV fragment of TNF $\alpha$  that binds to a TNF $\alpha$  receptor.

15. (Cancelled)

16. (Withdrawn) The chimeric TNFa of claim 14 that is less susceptible to cleavage from the surface of cells than native TNFa.

17. (Withdrawn) The chimeric TNFa of claim 16, wherein the cleavage rate of the chimeric TNFa is at least 90% less than that of native TNFa.

18. (Withdrawn) The chimeric TNFa of claim 14, further comprising a Domain II fragment of the other tumor necrosis factor ligand.

19. (Withdrawn) The chimeric TNFa of claims 14 or 18, further comprising a Domain I fragment of the other tumor necrosis factor ligand.

20. (Withdrawn) The chimeric TNFa of claims 14, 18 or 19, further comprising a fourth Domain IV fragment of the other tumor necrosis factor ligand.

21. (Withdrawn) The chimeric TNFa of claim 14, wherein the other tumor necrosis factor ligand is selected from the group consisting of CD154, CD70, Fas ligand, NGF, CD30, TNF $\beta$ , 4-1BBL and TRAIL.

22. (Cancelled)

23. (Withdrawn) The chimeric TNFa of claim 14, wherein the Domain IV fragment lacks a cleavage site of TNFa protein.

24. (Withdrawn) The chimeric TNFa of claim 14, comprising domains I, II and III, of a tumor necrosis factor ligand selected from the group consisting of CD154, CD70, Fas ligand, NGF, CD30, TNF $\beta$ , 4-1BBL and TRAIL, and domain IV of TNFa protein.

25. (Withdrawn) The chimeric TNFa of claim wherein one or more of the domains I, II and III are of CD154 protein.

26. (Withdrawn) The chimeric TNFa of claim 14, further comprising a linker domain encoding a peptide of at least one amino acid that links the Domain III fragment to the Domain IV fragment.

27. (Previously Presented) An expression vector, comprising the nucleic acid molecule of claim 2.

28. (Original) An expression vector, comprising the nucleic acid molecule of claim 3.

29. (Previously Presented) An expression vector, comprising the nucleic acid molecule of claim 4.

30-31. (Cancelled)

32. (Original) The expression vector of claim 27, further comprising viral DNA or bacterial DNA.

33. (Previously Presented) The expression vector of claim 32, wherein said viral DNA is selected from the group consisting of adenoviral DNA, retroviral DNA, or retroviral RNA.

34. (Previously Presented) The expression vector of claim 32, wherein at least a portion of the vector comprises adeno-associated viral DNA.

35. (Original) The expression vector of claim 27, further comprising a promoter region.

36. (Original) The expression vector of claim 27, further comprising a polyadenylation signal region.

37. (Previously Presented) A genetic construct comprising the nucleic acid molecule according to claim 2 operatively linked to a promoter sequence and to a polyadenylation signal sequence.

38. (Original) A host cell, comprising an expression vector according to claim 27 or a genetic construct according to claim 37.

39. (Original) The host cell of claim 38, wherein the cell is a mammalian cell.

40. (Original) The host cell of claim 39, wherein the cell is a tumor cell.

41. (Original) The host cell of claim 39, wherein the cell is an antigen presenting cell.

42. (Cancelled)

43. (Withdrawn) A method for increasing the concentration of a ligand capable of binding to a TNFa receptor on the surface of a cell, comprising introducing into the cell a nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 2, whereby the chimeric TNFa polypeptide is less susceptible to cleavage from the surface of the cells than a TNFa protein.

44. (Withdrawn) The method of claim 43, wherein the comprises an expression vector according to claim 27 or a genetic construct according to claim 37.

45. (Withdrawn) The method of claim 44 wherein the cell is a mammalian cell.

46. (Withdrawn) The method of claim 44 wherein the cell expresses a TNFa receptor on its surface.

47. (Withdrawn) A method for inducing apoptosis of a cell expressing a TNFa receptor, comprising introducing into the cell an encoding a chimeric TNFa polypeptide according to claim 1 or claim 2 wherein the chimeric TNFa polypeptide is expressed on the surface of the cell.

48. (Withdrawn) A method for inducing activation of an immune system cell, comprising introducing into the cell a nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 2 wherein the chimeric TNFa polypeptide is expressed on the surface of the cell.

49. (Withdrawn) A method for treating neoplasia in a patient comprising introducing into a neoplastic cell a nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 2 wherein the chimeric TNFa polypeptide is expressed on the surface of the cell.

50. (Withdrawn) The method of claim 49 further comprising: obtaining the neoplastic cell from a human patient; infusing the neoplastic cell back into the patient after having introduced into the cells the nucleic acid molecule encoding the chimeric TNFa polypeptide.

51. (Withdrawn) A method of treating neoplasia comprising directly injecting into a tumor bed of a patient the nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 2 wherein the chimeric TNFa polypeptide is expressed in the tumor bed.

52-61. (Cancelled)

62. (Withdrawn) A chimeric TNF $\alpha$  ligand polypeptide, comprising a Domain III fragment of a tumor necrosis factor ligand other than TNF $\alpha$ , wherein the fragment is a homolog of a cleavage site of native TNF $\alpha$ , and a Domain IV fragment of TNF $\alpha$  protein that binds to a TNF $\alpha$  receptor.

63. (Withdrawn) A method for inducing apoptosis of a cell expressing a TNF $\alpha$  receptor, comprising introducing into the cell an encoding a chimeric TNF $\alpha$  polypeptide according to claim 52 wherein the chimeric TNF $\alpha$  polypeptide is expressed on the surface of the cell.

64. (Withdrawn) A method for inducing activation of an immune system cell, comprising introducing into the cell a nucleic acid molecule encoding a chimeric TNF $\alpha$  polypeptide according to claim 52 wherein the chimeric TNF $\alpha$  polypeptide is expressed on the surface of the cell.

65. (Withdrawn) A method for treating neoplasia in a patient comprising introducing into a neoplastic cell a nucleic acid molecule encoding a chimeric TNF $\alpha$  polypeptide according to claim 52 wherein the chimeric TNF $\alpha$  polypeptide is expressed on the surface of the cell.

66. (Withdrawn) The method of claim 65 further comprising: obtaining the neoplastic cell from a human patient; infusing the neoplastic cell back into the patient after having introduced into the cells the nucleic acid molecule encoding the chimeric TNF $\alpha$  polypeptide.

67. (Withdrawn) A method of treating neoplasia comprising directly injecting into a tumor bed of a patient the nucleic acid molecule encoding a chimeric TNF $\alpha$  according to claim 52 wherein the chimeric TNF $\alpha$  polypeptide is expressed in the tumor bed.

68. (Previously Presented) A process for producing a chimeric TNF $\alpha$  ligand polypeptide of claim 2 comprising culturing a host cell of claim 38 under conditions suitable to effect expression of the protein.

69 -75. (Cancelled)

76. (New) The nucleic acid molecule according to Claim 2, wherein the encoded chimeric polypeptide is about 90% more resistant to cell membrane cleavage into soluble TNF $\alpha$  than are native TNF $\alpha$  and TNF $\alpha$  lacking the metalloproteinase cleavage site present from Val77 to Pro88 of native TNF $\alpha$ .

77. (New) An expression vector, comprising the nucleic acid molecule of Claim 71.

78. (New) A genetic construct, comprising the nucleic acid molecule of Claim 71 operatively linked to a promoter sequence and to a polyadenylation signal sequence.

79. (New) A host cell, comprising the expression vector of Claim 72 or the genetic construct of Claim 73.